REMARKS

I. PRELIMINARY COMMENTS

Applicant wishes to thank the Examiner for the courtesy shown the undersigned attorney during the brief telephonic discussion conducted March 12, 2003 at which the enclosed amendments were discussed. It is believed that these amendments resolve the outstanding issues under 35 U.S.C. §§ 112 and 102 and allow the Examiner to focus on any remaining issued under 35 U.S.C. § 103.

Thus, Claims 1, 14, 17, 18 and 20 have been amended consistent with suggestions of the Examiner in order to simplify the issues presented and to place those claims in condition for allowance. In particular, claim 1 has been amended to specifically recite that the analyte is an intracellular analyte so as to further distinguish that claim from the disclosure of Khanna et al. The amendments are supported by the specification as originally filed such as at page 10, lines 19-23 and page 12, lines 5-14 and do not introduce new matter into the specification.

The present invention is directed to specific binding assays in which the presence of an intracellular analyte in a sample is assayed for by steps including reaction of a specific binding partner for the analyte with the analyte to form a specific binding partner-analyte complex and detection of that complex. (One example of a specific binding partner-analyte complex is the complex formed between an antigen and an antibody specific for that antigen.) More particularly, the method of the invention includes the steps of mixing a sample of cells with a cell lysis agent to provide a lysed cellular sample, mixing the lysed cellular sample with a cyclodextrin sequestrant for the cell lysis reagent, and performing the specific binding assay in the presence of that sequestrant. The purpose of the

sequestrant is to prevent the cell lysis reagent from adversely affecting the binding reaction between the analyte and its specific binding partner.

II. OUTSTANDING REJECTIONS

Claims 1-2, 4-13, 16-19 and Claim 21 stand rejected under 35 U.S.C. §112 (second paragraph) as being indefinite.

Claims 1-2, 4-5, 8, 10, 14, 16-18 and 20 stand rejected under 35 U.S.C. §102(b) over Khanna, U.S. Patent No. 5,032,503.

Claims 7, 11-13 and 19 stand rejected under 35 U.S.C. §103(a) over Khanna, U.S. Patent No. 5,032,503 in view of Brown et al., U.S. Patent No. 5,739,001.

Claims 6 and 9 stand rejected under 35 U.S.C. §103(a) over Khanna, U.S. Patent No. 5,032,503 in view of Cook WO 94/26413.

Claim 21 stands rejected under 35 U.S.C. §103(a) over Khanna, U.S. Patent No. 5,032,503 in view of Edmonds, U.S. Patent No. 6,159,750.

III. PATENTABILITY ARGUMENTS

A. The Rejection Under 35 U.S.C. §112 (Second Paragraph) Should Be Withdrawn

The rejection under 35 U.S.C. §112 (second paragraph) should be withdrawn in light of the amendment of the claims.

B. The Anticipation Rejection Under 35 U.S.C. §102(b) Over Khanna Should Be Withdrawn

The anticipation rejection of claims 1-2, 4-5, 8, 10, 14, 16-18, and 20 under 35 U.S.C. §102(b) by Khanna (U.S. Patent No. 5,032,503) should be withdrawn on the

grounds that while it appears to be based on a theory of inherent anticipation it does not meet the elements for such anticipation. Specifically, while it might be possible for the detergent present in the ß-galactosidase enzyme reagent to lyse cells later in the Khanna process such lysis is by no means certain to occur. In fact, there is no teaching or suggestion that a cell lysis step be carried out and in the absence of such an instruction no guarantee that sufficient amounts or types of detergents would be applied to carry out the claimed step. While inherency is an accepted basis for rejection the law is clear that a recited element must necessarily be performed by the cited reference. In re Robertson, USPQ 2d 1949, 1950-51 (Fed. Cir. 1999) ("Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.") In the case of Khanna there is not such guarantee that cell lysis would invariably occur and as a result the anticipation rejection over that reference should be withdrawn.

Nevertheless, while Applicant disagrees with the outstanding rejection under 35 U.S.C. §102(b) they make the preceding amendment in accordance with the suggestion of the Examiner which recites that the analyte is an intracellular analyte released from lysis of the cells. While not conceding the patentability of the previous claims this new amendment is made in order to clearly eliminate the issue of (inherent) anticipation in order that the more important issue of what the references actually teach (obviousness) may be directly addressed.

C. The Rejections Under 35 U.S.C. §103(a) Over Khanna In View of Brown Should Be Withdrawn and No New Obviousness Rejections Should be Entered.

As discussed above, the anticipation rejections of claims 1-2, 4-5, 8, 10, 14, 16-18, and 20 over Khanna should be withdrawn in light of the amendment of claim 1 to distinguish over that reference. The separate issue then remains of whether those claims are obvious over the teachings of Khanna either alone or in combination with other references. It should be emphasized that the issues of (inherent) anticipation and obviousness are quite different ones and that even if it is accepted that Applicant's prior claims lacked novelty (which Applicant does not concede) there is no teaching in the cited references that renders those claims obvious under 35 U.S.C. 103.

The present invention, is directed to a method including the step of mixing a sample of cells with a cell lysis reagent (e.g. a detergent) as a sample pre-treatment method before assaying for an analyte.

Thus, even if it is accepted that the Khanna detergent which is used to dissociate a complex between the complementary members of the ß-galactosidase enzyme in the assay reagent could theoretically lyse sample cells there is no teaching in Khanna to i) carry out a cell lysis step, to ii) mix and react the lysed sample with a specific binding assay reagent for an intracellular analyte and to mix the lysed cellular sample with a cyclodextrin sequestrant for the cell lysis reagent.

It is true that Khanna "does not exclude...[pre-treatment of]...the sample at specific concentrations effective to specifically cause the cells in the sample to lyse" but with respect to an obviousness analysis the appropriate test is whether the references teach the claimed invention. As discussed at the Interview, Khanna teaches a dilution of the

surfactant containing solution prior to or upon its being contacted with the cyclodextrin component. Thus, the Khanna examples show a roughly two-fold dilution of the surfactant upon contacting with the cyclodextrin containing common reagent solution which further results in a solution having a lower surfactant concentration than those of skill in the art would be inclined to use to carry out cell lysis. Thus, there is no suggestion that use of a cyclodextrin sequestrant would reverse the negative effects of a cell lysis reagent according to Applicant's invention

Further, there is no such teaching in Khanna or any of the other cited references. Moreover, Khanna teaches away from cell lysis as a pre-treatment step when it is further stated that "sample pre-treatment will follow conventional procedures" (page 3, lines 19-20), and 'in the case of physiological fluids, other than the removal of particulates, no pre-treatment of the sample will usually be performed for purposes of the instant assay method' (column 5, lines 43-46). For this reason it is thus clear that Khanna not only fails to disclose the step of mixing a sample of cells with a detergent prior to assaying, but it actually teaches away from such a step.

Moreover, Khanna also teaches away from the use of levels of surfactant sufficient to lyse cells when it instructs that "[d]esirably, the concentration [of surfactant] will be insufficient to denature the specific binding pair members the sample analyte or any other assay reagents." (Col. 4, lines 8-11) This instruction to use minimal amounts of surfactants is contrary to the requirement of Applicant's invention that sufficient amounts of surfactant be used so as to rupture and permeate a cell wall so as to effect cell lysis. These amounts are typically 1%, as used in Applicant's examples, or greater. For these reasons,

no rejection under 35 U.S.C. §103(a) should be entered against any of claims 1, 2, 4-14 and 16-21.

Moreover, the rejection of claims 7, 11-13 and 19 under 35 U.S.C. §103(a) as being unpatentable over Khanna in view of Brown, U.S. Patent No. 5,739,001 should be withdrawn and no similar rejection should be made of the remaining claims over the references because Brown should not properly be combined with Khanna and does not make up for the deficiencies in Khanna, even if they were combined. Specifically, Brown discloses a "solid phase one-well cell based assay" which allows the study of biochemical processes in living cells responding to a stimulus by evaluating cell-related analytes (i) without the need to attach cells to the solid phase, (ii) without the need to employ cell culture techniques, and (iii) without the need to radioactively label the cell (column 3, lines 46-51). The assay disclosed in Brown involves lysis of the cell with a detergent (e.g. Triton X-100) but does not involve sequestration of the cell lysis reagent with a cyclodextrin. In fact, Brown is completely silent with regard to any adverse effects that the cell lysis reagent (e.g. Triton X-100) may have upon the binding reaction of the analyte to its specific binding partner. Apropos this, Applicant has now amended their claims to recite this feature as suggested by the Examiner.

As discussed above, Khanna describes the use of a surfactant to inhibit complex formation between complementary members of a specific binding pair (and not as a cell lysis reagent), and the addition of a cyclodextrin to neutralise the surfactant and thus initiate complex formation. The concentrations of surfactant cited in Khanna to inhibit binding range from 0.2-1.0% (Column 4, lines 13-19). In contrast, the highest concentrations of

surfactant used in the 'detection mix' of Brown (Column 7, lines 1-12), prior to dilution in the cell/stimulation media, is only 0.35%.

It is submitted that there is no motivation for the skilled person to combine the teachings of Brown with those of Khanna because they relate to different and unrelated concepts (i.e., cell lysis versus keeping binding partners apart). Furthermore, the skilled person would not be led to even consider the problems caused by the use of surfactants in the assay of Brown by the teachings of Khanna because of the much higher concentrations used therein. Therefore the skilled person would have no motivation to contemplate the use of cyclodextrin in the assay of Brown to overcome the problems addressed by the present invention. Accordingly, the rejection of claims 7, 11-13, and 19 under 35 U.S.C. §103(a) should be withdrawn.

D. The Rejection of Claims 6 and 9 Under 35 U.S.C. §103(a) Over Khanna and Cook Should Be Withdrawn

The rejection of claims 6 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Khanna in view of Cook (WO 94/26413) should be withdrawn because the person skilled in the art would not be motivated to incorporate the multiwell system described in Cook into the present invention and further because Cook fails to make up for the deficiencies in the disclosure of Khanna discussed above. Cook discloses an apparatus and method for studying cellular processes in intact cells using scintillation proximity assays. The apparatus comprises a vessel having a scintillant base which is adapted for the growth and attachment of cells. The method comprises introducing into a fluid suspension of cells a radiolabelled reagent under conditions to cause a portion of the labelled reagent to become associated with or released from the cells in order to study the cellular process.

Specifically, an essential feature of the present invention is the ability to lyse cells within the assay dish, then utilize cyclodextrin to neutralize the cell lysis reagent to enhance specific binding reactions. The main focus of the multiwell system in Cook is that the scintillant incorporated into the plastic allows the cells to remain intact. As a result, Cook teaches away from the need to disrupt cells in order to measure cellular analytes.

As discussed above, Khanna does not describe a pre-treatment lysis method. Furthermore, neither Cook nor Khanna teach lysis of cells with a detergent. Thus the combination of Khanna with Cook would not lead the skilled person to the present invention where cells are cultured, lysed and assayed in a single reaction vessel.

E. The Rejection of Claim 21 Under 35 U.S.C. §103(a) Over Khanna and Edmonds Should Be Withdrawn

The rejection of claim 21 over Khanna in view of Edmonds U.S. Patent No. 6,159,750 should be withdrawn. Edmonds teaches a method of fluorescence polarisation immunoassay which involves detecting and measuring the concentration of an analyte in a sample. Applicant understands that Edmonds is only cited for the disclosure of using fluorescence polarization but the patentability of claim 21 rests, in part, on the patentability from which it depends. Thus, it is relevant that Edmonds fails to make up for the deficiencies of Khanna in teaching the subject matter of independent claim 1.

Specifically, nowhere within Edmonds is there any disclosure of a sample pre-treatment to lyse intact cells prior to the determination of the analyte concentration. Furthermore, the patent is completely silent with regards to the use of any sample pre-treatment, other than "diluents or analytical reagents" (Column 4, lines 18-19). In fact, although there is reference to the sample being "a body fluid such as blood, serum, plasma, or urine etc." (Column 4, lines 35-36), there is no mention of the method being applicable

for use in "a cell sample." Accordingly, Edmonds fails to make up for the deficiencies in Khanna which also fails to disclose the step of mixing a sample of cells with a cell lysis reagent prior to assaying. Thus a combination of the teachings of Edmonds with those of Khanna would not lead the skilled person to arrive at the present invention, which is inventive thereover and claim 21 should be allowed.

CONCLUSION

For the foregoing reasons, the rejections should be withdrawn and all claims 1, 2, 4-14, and 16-21 should be allowed. Should the Examiner wish to discuss any issues of form or substance in order to expedite allowance of the pending application, she is invited to contact the undersigned attorney at the number indicated below.

Attached hereto are the changes made to the claims by the current amendments. The attached page is captioned "Version with Markings to Show Changes Made."

The Commissioner is authorized to charge any fee deficiency required by this paper to Deposit Account No. 13-2855.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

1. [FIVE TIMES AMENDED] An improved method of conducting a specific binding assay for the presence of an intracellular analyte in a cultured cell sample which method comprises the steps of:

i) mixing a sample of cultured cells with a cell lysis reagent to provide a lysed cellular sample;

ii) mixing and reacting the lysed cellular sample with a specific binding assay reagent comprising a specific binding partner of the intracellular analyte and a tracer to perform a specific binding assay; [by] thus forming a reaction mixture comprising a specific-binding partner-intracellular analyte complex;

iii) mixing the lysed cellular sample with a cyclodextrin sequestrant for the cell lysis reagent, whereby the specific binding assay of step ii) is performed in the presence of the sequestrant; and

iv) detecting the presence of the specific binding partner-intracellular analyte complex, the presence of which is indicative of the presence of intracellular analyte in the sample wherein the improvement lies in the sequestrant preventing the cell lysis reagent from adversely affecting a binding reaction between the analyte and its specific binding partner.

14. [TWICE AMENDED] A kit, suitable for assaying for an analyte by the method as claimed in claim 17 [which method further comprises the step of separating bound tracer from unbound tracer,] comprising: a detergent; a sequestrant for the detergent; a specific binding partner of the analyte; a tracer; and separation means for separating bound tracer from unbound tracer.

17. [TWICE AMENDED] The method as claimed in claim 1, which further comprises the step of separating bound tracer from unbound tracer [wherein the specific binding partner is labelled with a tracer].

18. [THREE TIMES AMENDED] The method as claimed in claim 1, wherein the [assay reagent] tracer [label for detection wherein the label] is selected from the group consisting of radioactive isotope label, enzyme-linked label and fluorescent label.

20. [AMENDED] A kit suitable for assaying for an analyte by the method claimed in Claim 1, comprising a detergent, a cyclodextrin sequestrant for the detergent, a specific binding partner for the analyte and a vessel suitable for cell culture.